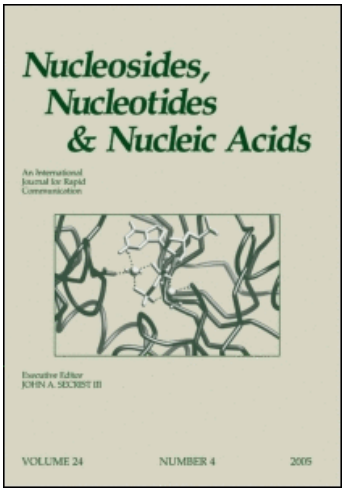


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Nucleosides and Nucleotides. 192. Toward the Total Synthesis of Cyclic ADP-Carbocyclic-Ribose. Formation of the Intramolecular Pyrophosphate Linkage by a Conformation-Restriction Strategy in a *Syn*-form Using a Halogen Substitution at the 8-Position of the Adenine Ring

Yuji Sumita[a]; Michiyo Shirato[a]; Yoshihito Ueno[a]; Akira Matsuda[a]; Satoshi Shuto[a]
[a] Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

To cite this Article Sumita, Yuji , Shirato, Michiyo , Ueno, Yoshihito , Matsuda, Akira and Shuto, Satoshi(2000) 'Nucleosides and Nucleotides. 192. Toward the Total Synthesis of Cyclic ADP-Carbocyclic-Ribose. Formation of the Intramolecular Pyrophosphate Linkage by a Conformation-Restriction Strategy in a *Syn*-form Using a Halogen Substitution at the 8-Position of the Adenine Ring', Nucleosides, Nucleotides and Nucleic Acids, 19: 1, 175 — 187
To link to this Article: DOI: 10.1080/15257770008033002
URL: http://dx.doi.org/10.1080/15257770008033002

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(1&2), 175–187 (2000)

Nucleosides and Nucleotides. 192. Toward the Total Synthesis of Cyclic ADP-Carbocyclic-Ribose. Formation of the Intramolecular Pyrophosphate Linkage by a Conformation-Restriction Strategy in a *Syn*-form Using a Halogen Substitution at the 8-Position of the Adenine Ring[1]

Yuji SUMITA, Michiyo SHIRATO, Yoshihito UENO, Akira MATSUDA, and Satoshi SHUTO*

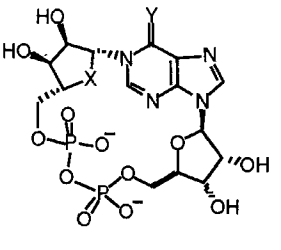
Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Dedicated to the memory of Dr. Gertrude. B. Elion

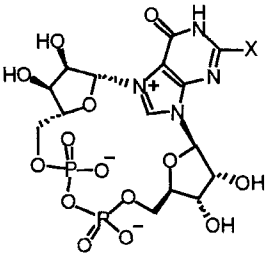
Abstract: The synthesis of cyclic ADP-carbocyclic-ribose (**2**), as a stable mimic for cyclic ADP-ribose, was investigated. Construction of the 18-membered backbone structure was successfully achieved by condensation of the two phosphate groups of **19**, possibly due to restriction of the conformation of the substrate in a *syn*-form using an 8-chloro substituent at the adenine moiety. SN2 reactions between an optically active carbocyclic unit **8**, which was constructed by a previously developed method, and 8-bromo-N^6-trichloroacetyl-2',3'-*O*-isopropylideneadenosine **9c** gave *N*-1-carbocyclic derivative, which was deprotected to give 5',5"-diol derivatives **18**. When **18** was treated with $POCl_3$ in $PO(OEt)_3$, the bromo group at the 8-position was replaced to give *N*-1-carbocyclic-8-chloroadenosine 5',5"-diphosphate derivative **19** in 43% yield. Treatment of **19** with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride gave the desired intramolecular condensation product **20** in 10% yield. This is the first chemical construction of the 18-membered backbone structure containing an intramolecular pyrophosphate linkage of a cADPR-related compound with an adenine base.

Cyclic ADP-ribose (cADPR, **1**, FIG. 1) is a newly discovered general mediator involved in Ca^{2+} signaling.[2] Due to their biological importance, the synthesis of cADPR analogs has been extensively studied by enzymatic and chemo-enzymatic methods using ADP-ribosyl cyclase.[3] ADP-ribosyl cyclase from *Aplysia California* mediates the intramolecular ribosylation of NAD^+ and some modified NAD^+ analogs, which are prepared chemically or enzymatically, at the *N*-1-position of the purine moiety, to yield cADPR or the

FIG. 1



1 (cADPR): X = O, Y = NH
2: X = CH_2, Y = NH
3: X = CH_2, Y = O

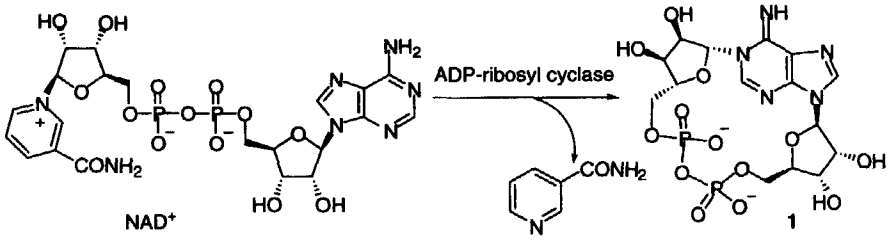


4: X = H
5: X = NH_2

*Tel: +81-11-706-3228, Fax: +81-11-706-4980, e-mail: shu@pharm.hokudai.ac.jp

corresponding analogues (Scheme 1).[3] However, the analogues that can be obtained by this method are limited due to the substrate specificity of the enzyme. Furthermore, even though ADP-ribosyl cyclase catalyzes the cyclization of NAD^+ analogs, in some cases the newly formed glycosyl bond is attached to the *N*-7 nitrogen of the purine ring: e.g. the enzymatic reaction product of inosine or guanosine analog of NAD^+ is not the desired *N*-1-cyclized product, but rather the *N*-7-cyclized products **4** and **5**.[3g] Accordingly, the development of flexible methods for synthesizing cADPR and a variety of its analogs is urgently awaited.

Scheme 1



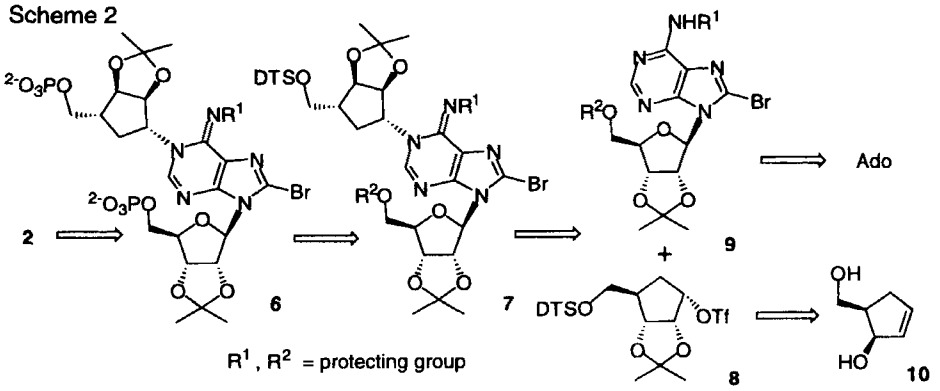
In cells, cADPR is synthesized from NAD^+ by ADP-ribosyl cyclase and acts as a transient second messenger; it is hydrolyzed promptly by cADPR hydrolase to give ADP-ribose and inactivated under physiological conditions.[2] cADPR is also known to be readily hydrolyzed non-enzymatically at the unstable *N*-1-glycosidic linkage of its adenine moiety to give ADP-ribose, even in neutral aqueous solution.[2d] Although further intensive studies of cADPR are needed because of its biological importance, this biological as well as chemical instability of cADPR limits studies of its physiological role, at least to some extent. Therefore, stable analogues of cADPR that exhibit Ca^{2+}-mobilizing activity in cells similar to that of cADPR are urgently required.

We designed cyclic ADP-carbocyclic-ribose (**2**) and its inosine congener **3** (cIDP-carbocyclic-ribose, FIG. 1)[4] as stable mimics of cADPR, in which an oxygen atom in the ribose ring of cADPR is replaced by a methylene group. The mimics **2** and **3** should be resistant to both enzymatic and chemical hydrolysis, since they lack the unstable *N*-1-glycosidic linkage of cADPR. These analogs preserve all of the functional groups of cADPR, except for this ring oxygen, and these molecules should have a conformation similar to that of cADPR. Therefore, we expect that these analogs would effectively mobilize intracellular Ca^{2+}, like cADPR, so that they could be used as pharmacological tools for studying the mechanism of cADPR- modulated Ca^{2+}-signaling pathways. We previously achieved the synthesis of the inosine congener **3**,[4] which is the first chemical synthesis of a cADPR analog[5] and may lead to the development of general methods for synthesizing cyclic nucleotides of this type. In this report, we describe the results of a synthetic study toward another target, cADP-carbocyclic ribose (**2**).

The plan for synthesizing **2** is shown in Scheme 2, and is similar to that of our previous synthesis of the inosine congener **3**. Cyclization of the 18-membered ring to form a pyrophosphate linkage is carried out by intramolecular condensation between the two phosphate groups of *N*-1-carbocyclic-8-bromoadenosine diphosphate derivative **6**, which can be prepared from the *N*-1-(carbocyclic-ribosyl)-8-bromoadenosine derivative **7**. Compound **7** would be obtained in an SN2 reaction between carbocyclic unit **8** and the protected 8-bromoadenosine

derivative **9**, which is prepared from adenosine (Ado). The carbocyclic unit **8**, which was also used previously in the synthesis of **3**, is prepared from optically active cyclopentene derivative **10**.[4]

Scheme 2



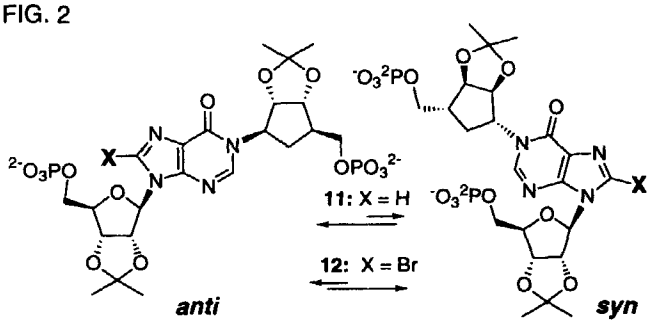
The condensation reaction between the two phosphate groups to form an intramolecular pyrophosphate linkage is the key step in the chemical synthesis of cADPR, and we as well as other groups have experienced that this key step is very difficult to achieve.[4-6] Therefore, the development of an efficient method for forming the intramolecular pyrophosphate linkage should greatly promote progress in this research area.

During previous synthetic studies on **3**, we found that the *syn-anti* conformations around the glycosidic bond of the molecule are important with regard to whether or not the intramolecular condensation reaction between the two phosphate groups occurs (FIG. 2).[7-9] Introducing a bulky substituent to the 8-position of purine nucleosides is known to restrict the conformation in a *syn*-form.[7-9] Therefore, we used an 8-bromo-substituted inosine analog **12** for the intramolecular condensation reaction, and in fact, the key intramolecular condensation reaction proceeded only when a bromo-substituent was introduced at the 8-position of the hypoxanthine ring of the substrate, and achieved the synthesis of **3**. Based on these findings, we planned a synthetic scheme using 8-bromoadenosine derivative **9** as an adenine nucleoside unit, shown in Scheme 2.

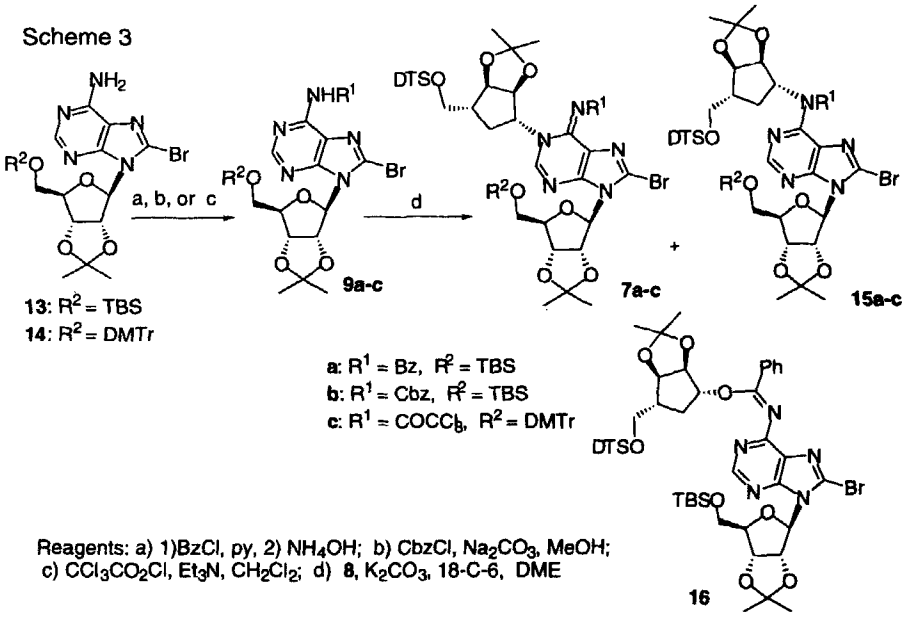
Another problem in this synthesis is the need to construct an *N*-1-carbocyclic-ribosyladenosine structure such as **7**. When adenosine derivative **13** is treated with carbocyclic triflate **8**, we would anticipate that not the desired *N*-1-carbocyclic product but rather the corresponding N^6-product may be the main product. Therefore, protection of the 6-amino group would be needed considering the reactions described in Scheme 2. The *N*-1/N^6-regioselectivity in the reaction of **8** with **9** would change depending on the protecting group introduced at the N^6-position.

We first examined the reaction with a benzoyl (Bz) group, which is frequently used to protect the 6-amino group of adenosine (Scheme 3). When N^6-benzoyl-8-bromoadenosine derivative **9a**, prepared from 8-bromo-2',3'-*O*-isopropylidene-5'-*O*-TBS-adenosine (**13**), was treated with carbocyclic triflate **8** in the presence of K_2CO_3 in DME, the desired *N*-1 product **7a** was not obtained at all. In this reaction, the carbocyclic unit was substituted at the N^6-benzoyl moiety to give N^6-substituted product **15a** and N^6-methylidene-type product **16** in yields of 22% and 4%, respectively.[10]

FIG. 2



Scheme 3

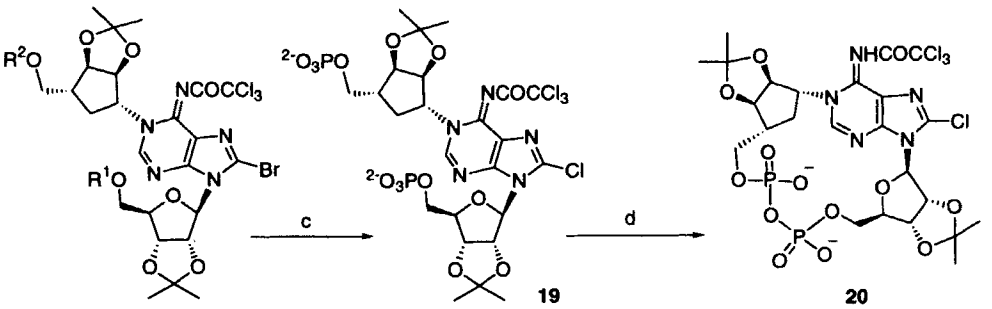


Reagents: a) 1)BzCl, py, 2) NH_4OH; b) CbzCl, Na_2CO_3, MeOH; c) CCl_3CO_2Cl, Et_3N, CH_2Cl_2; d) **8**, K_2CO_3, 18-C-6, DME

A benzyloxycarbonyl (Cbz) group was next examined as a protecting group. However, the yield of the introduction of a Cbz group at the N^6 position was low, and the reaction of the resulting N^6-Cbz derivative **7b** with **8** gave N^6-carbocyclic product **15b** as a sole product.

Based on these results, we presumed that introduction of a rather electron-withdrawing protecting group at the amino group would decrease the nucleophilicity of the N^6 position, and therefore the desired *N*-1 product might be obtained predominantly (Scheme 4). Thus, we selected a trichloroacetyl group as a protecting group, and 8-bromo-2',3'-*O*-isopropylidene-5'-*O*-dimethoxytrityladenosine (**14**) was treated with CCl_3COCl and Et_3N in dichloroethane at 0 °C to give the corresponding N^6-trichloroacetyl derivative **9c** as a major product. However, this was unstable and immediately used for the next reaction with the carbocyclic unit **8** without purification. When **9c** was heated with the carbocyclic unit **8** at 50 °C in the presence of K_2CO_3 and 18-crown-6 in DME, the desired *N*-1-carbocyclic product **7c** was obtained in 19% yield, and **14** was recovered in 61% yield after silica gel column chromatography. The regio- and

Scheme 4



a 7c: R^1 = DMTr, R^2 = DTS
a 17: R^1 = H, R^2 = DTS
b 18: R^1 = R^2 = H

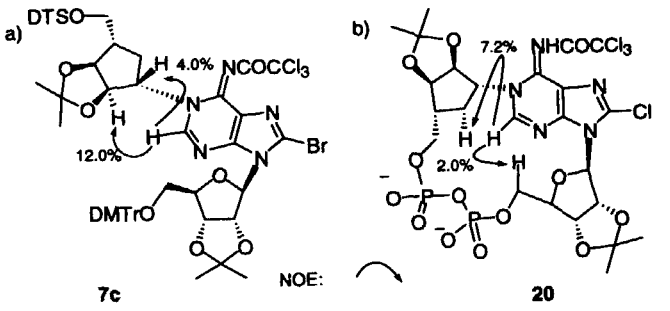
Reagents: a) AcOH, THF; b) TBAF, AcOH, THF; c) $POCl_3$, $PO(OEt)_3$; d) EDC, NMP

stereochemistries of **7c** were confirmed by NOE and HMBC experiments. When H-2 of **7c** was irradiated, 4.0% and 12.0% NOEs at H-2" and H-1" of the carbocyclic moiety were observed (FIG. 4a). The HMBC spectrum showed a correlation between C-2 of the adenine moiety and H-1" of the carbocyclic moiety. Although the yield was insufficient, this is the best result in our investigation for obtaining an *N*-1-carbocyclic-8-bromoadenosine derivative.

The DMTr group of **7c** was removed with AcOH/THF to give **17** in 64% yield, which was further treated with TBAF/AcOH/THF to give diol **18** in high yield. The introduction of two phosphate groups at the 5' and 5"-hydroxyls was examined next. We previously used a phosphoramidite method with (2-cyanoethoxy)(*N,N*-diisopropylamino)chlorophosphine to introduce two phosphate groups in the synthesis of cIDP-cabocyclic-ribose (**3**).[4] Dimroth rearrangement might occur during the phosphoramidite procedure in this adenosine derivative, since it needs a treatment under basic conditions to remove the cyanoethyl group. Therefore, we used Yoshikawa's phosphorylation method[11] under rather acidic conditions. Treatment of **18** with $POCl_3$ in $PO(OEt)_3$ at 0 °C and subsequent DEAE-Sephadex column chromatography gave the diphosphate derivative **19** as a triethylammonium salt in 43% yield. However, a molecular-ion peak of this nucleotide observed at *m/z* 814.0003 (calcd for $C_{24}H_{30}ClN_5O_{14}P_2$, 814.0018) in high-resolution FABMS showed that the bromo group at the 8-position was replaced with a chloro group during the phosphorylation reaction.

We attempted the condensation reaction between the two phosphate groups that formed an intramolecular pyrophosphate linkage with the 8-chloroadenosine diphosphate derivative **19**, since the chloro group, similar to a bromo group, should restrict the conformation around the glycosidic linkage and the *syn*-conformer in **19** would predominantly exist over the *anti*-conformer. Thus, the intramolecular condensation reaction of **19** was investigated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in *N*-methylpyrrolidone (NMP) under various conditions. Consequently, when **19** was heated with 2 equiv of EDC at 80 °C in NMP, the best result was obtained: the desired cyclic product was obtained after purification by ion-exchange column chromatography, although the yield was not sufficient (10%).[12] The cyclic structure of **20** was confirmed by the following data: 1) molecular-ion peaks

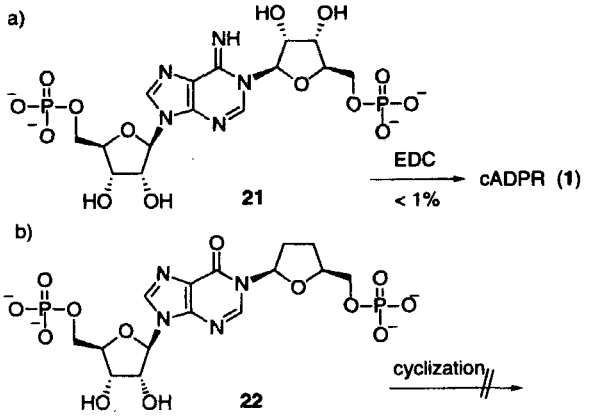
FIG. 4



corresponding to **20** were observed at *m/z* 796, 798, and 800 in a FAB mass spectrum; 2) its ^{31}P NMR spectrum showed two signals at -10.51 and -10.57 ppm, which are typical chemical shifts for a pyrophosphate moiety with a coupling constant (J = 12.5 Hz) similar to those of cADPR (-9.92 and -10.67 ppm, J = 14.6 Hz)[13] and **3** (-9.16 and -10.51, J = 10.7 Hz)[4]; 3) when H-2 of the adenine moiety was irradiated, NOEs were observed at H-5" of the carbocyclic moiety (7.2%) and H-5'of the ribose moiety (FIG. 4b), while such NOEs were not observed in uncyclized **19**.

Attempts to prepare cADPR or its analogs by chemical intramolecular condensation were first reported by Gu and Sih.[14] They investigated condensation between the two phosphate groups of *N*-1-phosphoribosyl-AMP (**21**) with EDC, but were unsuccessful (yield < 1%) (Scheme 5a).[13] Later, ring-closure of diphosphate **22** through the formation of a pyrophosphate linkage was examined by Potter and Fortt, but they failed (Scheme 5b).[5a] On the other hand, we succeeded in the cyclization of the diphosphate substrates, i.e. inosine derivative **12** and adenosine derivative **19**, which had a bromo or chloro substitution at the 8-position of the purine moiety. These results suggest that such a substituent at the purine-8-position facilitates the condensation reaction to form an intramolecular pyrophosphate linkage.

Scheme 5



In summary, we designed carbocyclic analog **2** as a stable mimic of cADPR, and successfully constructed its 18-membered backbone structure. This is the first chemical synthesis

of a cADPR-related compound with an adenine base. This study, as well as a previous synthetic study on cIDP-carbocyclic-ribose (**3**), has demonstrated that the 8-bromo or –chloro group in the purine moiety to facilitate the key intramolecular condensation reaction between the phosphate groups of *N*-1-(carbocyclic-ribosyl)purine nucleoside diphosphates. This is probably due to conformational restriction of the molecule in a *syn*-form around its glycosyl linkage.

Reductive removal of the 8-chloro group and deprotection of the 2',3'-*O*-isopropyridene group and the trichloroacetyl group are now under investigation.

Experimental Section

Melting points are uncorrected. NMR spectra were recorded at 270, 400, or 500 MHz (^{1}H), at 67.8 MHz (^{13}C), and 125 MHz (^{31}P), and the data assigned based on H-H and C-H COSY spectra are reported in ppm downfield from TMS (^{1}H and ^{13}C) or H_3PO_4 (^{31}P). Mass spectra were obtained by electron ionization (EI) or fast atom bombardment (FAB) method. Thin-layer chromatography was performed on Merck coated plate $60F_{254}$. Silica gel chromatography was performed with Merck silica gel 5715. Reactions were carried our under an argon atmosphere.

8-Bromo-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine (13). A mixture of 8-bromo-2',3'-*O*-isopropylideneadenosine[9] (4.04 g, 10.4 mmol), imidazole (2.31 g, 31.4 mmol), and TBSCl (3.10 g, 20.8 mmol) in DMF (100 mL) was stirred at room temperature for 3 h. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 1:1) to give **13** (4.67 g, 89%) as solids: UV (MeOH) λ_{max} 263 nm; EI-MS *m/z* 499 (M^+, 0.7%), 501 (M^+, 0.7%); EI-HRMS calcd for $C_{19}H_{30}BrN_5O_4Si$ 499.1251, found 499.1234; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 8.29 (s, 1 H, H-2), 6.19 (d, 1 H, H-1', J = 1.9 Hz), 5.80 (dd, 1 H, H-2', J = 1.9, 6.4 Hz), 5.56 (s, 2 H, -NH_2), 5.15 (dd, 1 H, H-3', J = 3.3, 6.4 Hz), 4.29 (ddd, 1 H, H-4', J = 3.3, 6.5, 6.8 Hz), 3.75 (dd, 1 H, H-5', J = 6.8, 10.7 Hz), 3.64 (dd, 1 H, H-5', J = 6.5, 10.7 Hz), 1.62, 1.42 (each s, each 3 H, *i*-Pr-Me), 0.84 (s, 9 H, *t*-Bu), 0.05 (s, 6 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 154.86, 153.35, 150.89, 128.27, 120.59, 114.43, 92.06, 88.66, 83.31, 82.61, 63.56, 27.62, 26.31, 26.27, 26.16, 25.91, 18.7.

8-Bromo-5'-*O*-(4,4'-dimethoxytrityl)-2',3'-*O*-isopropylideneadenosine (14). A mixture of 8-bromo-2',3'-*O*-isopropylideneadenosine[9] (6.0 g, 15.5 mmol) and DMTrCl (7.9 g, 23.3 mmol) in pyridine (60 mL) was stirred at room temperature for 7 h. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 1:2) to give **14** (7.2 g, 67%) as solids: UV (MeOH); λ_{max} 266 (sh 280), 237 nm; FAB-MS (positive) *m/z* 688 (MH^+, 7%), 690 (MH^+, 7%); FAB-HRMS calcd for $C_{34}H_{35}BrN_5O_6$ 688.1771, found 688.1779; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 8.00 (s, 1 H, H-2), 7.34-7.14 (m, 9 H, DMTr), 6.74-6.67 (m, 4 H, DMTr), 6.20 (d, 1 H, H-1', J = 1.3 Hz), 5.70 (dd, 1 H, H-2', J = 1.3, 5.9 Hz), 5.59 (s, 2 H, -NH_2), 5.11 (dd, 1 H, H-3', J = 3.3, 5.9 Hz), 4.51 (ddd, 1 H, H-4', J = 3.3, 5.9, 7.9 Hz), 3.77 (s, 6 H, OMe×2), 3.23 (dd, 1 H, H-5', J = 7.9, 9.9 Hz), 3.13 (dd, 1 H, H-5', J = 5.9, 9.9 Hz), 1.62, 1.38 (each s, each 3 H, *i*-Pr-Me); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 158.76, 154.63, 153.14, 150.69, 145.10, 136.35, 136.20, 130.35, 128.64, 128.48, 128.10, 128.05, 127.04, 120.47, 114.36, 113.35, 92.08, 88.07, 86.38, 83.61, 83.11, 64.48, 55.62, 27.57, 25.88.

N^6-Benzoyl-8-bromo-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine (9a). A mixture of **13** (10 g, 20.0 mmol) and BzCl (7.0 mL, 60.0 mmol) in pyridine (100 mL) was stirred at room temperature for 7 h. After ice was added, the resulting mixture was stirred at room temperature for 1 h, and then NH_4OH (28%, 8.0 mL) was added at 0 °C, which was further stirred at room temperature for 17 h. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 2:1) to give **9a** (9.4 g, 71%) as solids: UV (MeOH) λ_{max} 285 nm; EI-MS *m/z* 603 (M^+, 0.3%), 605 (M^+, 0.2%); EI-HRMS calcd for $C_{26}H_{34}BrN_5O_5Si$ 603.1513, found 603.1498; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 8.96 (s, 1 H, NH), 8.82 (s, 1 H, H-2), 8.06-7.55 (m, 5 H, phenyl), 6.29 (d, 1 H, H-1', J = 2.0 Hz), 5.86 (dd, 1 H, H-2', J = 2.0, 6.4 Hz), 5.22 (dd, 1 H, H-3', J = 3.4, 6.4 Hz), 4.36 (ddd, 1 H, H-4', J = 3.4, 6.4, 6.6 Hz), 3.81 (dd, 1 H, H-5', J = 6.6, 10.7 Hz), 3.71 (dd, 1 H, H-5', J = 6.4, 10.7 Hz), 1.68, 1.47 (each s, each 3 H, *i*-Pr-Me), 0.89 (s, 9 H, *t*-Bu), 0.01 (s, 6 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 164.38, 152.54, 151.97, 148.43, 133.46, 132.85, 131.39, 128.84, 127.80, 123.20, 114.23, 91.71, 88.09, 82.75, 81.89, 62.98, 27.17, 25.78, 25.41, 18.30. *Anal.* calcd for $C_{26}H_{34}BrN_5O_5Si \cdot 5/4H_2O$: C, 49.80; H, 5.87; N, 11.17. Found: C, 49.92; H, 5.51; N, 11.07.

N^6-Benzyloxycarbonyl-8-bromo-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-adenosine (9b). A mixture of **13** (500 mg, 1.0 mmol), Na_2CO_3 (530 mg, 5.0 mmol), and CbzCl (710 μL, 5.0 mmol) in MeOH (6 mL) was stirred at room temperature for 14 h. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water, 0.1 N HCl, and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 3:1) to give **9b** (126 mg, 20%) as solids: UV (MeOH) λ_{max} 273 (sh 280) nm; FAB-MS (positive) *m/z* 634 (MH^+, 97%), 636 (MH^+, 100%); FAB-HRMS calcd for $C_{27}H_{37}BrN_5O_6Si$ 634.1697, found 634.1676; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 8.71 (s, 1 H, H-2), 8.02 (s, 1 H, -N*H*-), 7.47-7.30 (m, 5 H, phenyl), 6.21 (d, 1 H, H-1', J = 2.0 Hz), 5.79 (dd, 1 H, H-2', J = 2.0, 6.4 Hz), 5.30 (s, 2 H, -CH_2-Ph), 5.16 (dd, 1 H, H-3', J = 3.5, 6.4 Hz), 4.30 (ddd, 1 H, H-4', J = 3.5, 6.4, 6.5 Hz), 3.74 (dd, 1 H, H-5', J = 6.4, 10.8 Hz), 3.64 (dd, 1 H, H-5', J = 6.5, 10.8 Hz), 1.63, 1.42 (each s, each 3 H, *i*-Pr-Me), 0.83 (s, 9 H, *t*-Bu), 0.06 (s, 6 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 152.67, 151.45, 150.42, 148.21, 135.17, 130.96, 128.55, 122.28, 114.18, 91.68, 88.12, 82.73, 81.89, 67.87, 62.97, 27.15, 25.77, 25.41, 18.28. *Anal.* calcd for $C_{27}H_{36}BrN_5O_6Si$: C, 51.10; H, 5.72; N, 11.04. Found: C, 51.27; H, 5.74; N, 11.13.

N^6-Benzoyl-8-bromo-N^6-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(dimethyl-thexylsilyloxymethyl)cyclopentyl]-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-adenosine (15a) and 8-Bromo-N^6-[1-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(dimethyl-thexylsilyloxymethyl)cyclopentyloxy]-1-phenylmethylidene]-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine (16). A mixture of **9a** (3.67 g, 6.06 mmol), 18-crown-6 (961 mg, 3.63 mmol), and K_2CO_3 (838 mg, 6.06 mmol) in DME (7 mL) was heated under reflux for 4 h. To the mixture was added a solution of **8** (2.57 g, 6.06 mmol) in DME (3 mL) at 50 °C, and the resulting mixture was stirred at the same temperature for 12 h. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 5:1 then 4:1) to give **15a** (1.23 g, 22%) as solids and **16** (211 mg, 4%) as solids. **15a**: UV (MeOH) λ_{max} 290 nm; FAB-MS (positive) *m/z* 916 (MH^+, 14%), 918 (MH^+, 15%);

FAB-HRMS calcd for $C_{43}H_{67}BrN_5O_8Si_2$ 916.3712, found 916.3712; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.56 (s, 1 H, H-2), 7.43-7.13 (m, 5 H, phenyl), 6.11 (d, 1 H, H-1', *J* = 2.0 Hz), 5.66 (dd, 1 H, H-2', *J* = 2.0, 6.2 Hz), 5.24 (dd, 1 H, H-2", *J* = 3.7, 6.5 Hz), 5.11 (m, 1 H, H-1"), 5.08 (dd, 1 H, H-3', *J* = 3.7, 6.2 Hz), 4.58 (m, 1 H, H-3"), 4.23 (ddd, 1 H, H-4', *J* = 3.2, 5.7, 6.3 Hz), 3.79 (dd, 1 H, H-6", *J* = 3.5, 9.8 Hz), 3.68 (dd, 1 H, H-5', *J* = 6.3, 10.8 Hz), 3.64 (dd, 1 H, H-5', *J* = 5.7, 10.8 Hz), 3.62 (dd, 1 H, H-6", *J* = 1.9, 9.8 Hz), 2.38-2.24 (m, 3 H, H-5"×2, H-4"), 1.60 (m, 1 H, thexyl-CH), 1.56, 1.49, 1.38, 1.29 (each s, each 3 H, *i*-Pr-Me), 0.88-0.81 (s, 21 H, *t*-Bu, thexyl-Me), 0.06--0.08 (m, 12 H, SiMe) ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 171.79 (C=O), 153.58 (C6), 152.88 (C4), 151.81 (C2), 136.50, 132.31 (C8), 130.87 (C5), 128.70, 127.76, 114.34 (-*C*$(CH_3)_2$), 112.47 (-*C*$(CH_3)_2$), 91.41 (C1'), 87.67 (C4'), 83.72 (C2"), 82.55 (C2'), 81.65 (C3"), 81.10 (C3'), 65.03 (C1"), 63.80 (C6"), 62.91 (C5'), 47.06 (C4"), 34.16, 32.40 (C5"), 27.89, 27.21, 25.84, 25.61, 25.43, 25.09, 20.89, 18.51, -3.50. **16**: UV (MeOH) λ_{max} 272, 245 nm; FAB-MS (positive) *m/z* 916 (MH^+, 38%), 918 (MH^+, 39%); FAB-HRMS calcd for $C_{43}H_{67}BrN_5O_8Si_2$ 916.3712, found 916.3737; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ; 8.53 (s, 1 H, H-2), 7.38-7.20 (m, 5 H, phenyl), 6.18 (d, 1 H, H-1', *J* = 1.8 Hz), 5.77 (dd, 1 H, H-2', *J* = 1.8, 6.2 Hz), 5.54 (m, 1 H, H-1"), 5.14 (dd, 1 H, H-3', *J* = 3.4, 6.2 Hz), 4.85 (d, 1 H, H-2" or H-3", *J* = 5.8 Hz), 4.66 (d, 1 H, H-2" or H-3", *J* =5.8 Hz), 4.27 (ddd, 1 H, H-4', *J* = 3.4, 6.3, 6.7 Hz), 3.73 (dd, 1 H, H-5', *J* = 6.7, 10.6 Hz), 3.66 (dd, 1 H, H-5', *J* = 4.3, 10.6 Hz), 3.67-3.61 (m, 2 H, H-6"×2), 2.53-2.41 (m, 2 H, H-5", H-4"), 1.97 (m, 1 H, H-5"), 1.61 (m, 1 H, thexyl-CH), 1.58, 1.49, 1.41, 1.38 (each s, each 3 H, *i*-Pr-Me), 0.90-0.82 (s, 21 H, *t*-Bu, thexyl-Me), 0.08-0.05 (m, 12 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 160.99 (C=N), 157.52 (C6), 152.63 (C2), 152.02 (C4), 131.09 (C8), 130.91, 128.72, 128.18, 125.55 (C5), 114.11 (-*C*$(CH_3)_2$), 110.69 (-*C*$(CH_3)_2$), 91.48 (C1'), 87.92 (C4'), 84.76 (C2"), 83.18 (C1"), 82.70 (C2'), 82.14 (C3"), 81.96 (C3'), 63.65 (C6"), 63.02 (C5'), 47.10 (C4"), 34.20, 31.38 (C5"), 27.17, 26.67, 25.82, 25.62, 25.45, 25.14, 24.28, 20.36, 18.49, -3.50. *Anal.* calcd for $C_{43}H_{66}BrN_5O_8Si_2$: C, 56.32; H, 7.25; N, 7.64. Found: C, 56.31; H, 7.35; N 7.42.

N^6-Benzyloxycarbonyl-8-bromo-N^6-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(dimethylthexylsilyloxymethyl)cyclopentyl]-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine (15b). Compound **15b** (288 mg, 39%) was obtained as solids as described above for the reaction of **9a**, with **9b** (500 mg, 0.79 mmol) instead of **9a**, and **9b** (254 mg, 51%) was recovered, after silica gel column chromatography (hexane/EtOAc, 5:1 then EtOAc): UV (MeOH) λ_{max} 281 (sh 290) nm; FAB-MS (positive) *m/z* 946 (MH^+, 40%), 948 (MH^+, 43%); FAB-HRMS calcd for $C_{44}H_{69}BrN_5O_9Si_2$ 946.3817, found 946.3809; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1 H, H-2), 7.22-7.20 (m, 5 H, phenyl), 6.15 (d, 1 H, H-1', *J* = 1.8 Hz), 5.62 (dd, 1 H, H-2', *J* = 1.8, 6.2 Hz), 5.15 (s, 2 H, -CH_2-Ph), 5.08 (dd, 1 H, H-3', *J* = 3.7, 6.2 Hz), 5.03 (dd, 1 H, H-2", *J* = 4.2, 6.7 Hz), 4.83-4.79 (m, 1 H, H-1"), 4.37 (dd, 1 H, H-3", *J* = 6.5, 6.7 Hz), 4.21 (ddd, 1 H, H-4', *J* = 3.7, 6.2, 6.5 Hz), 3.67 (dd, 1 H, H-5', *J* = 6.5, 10.7 Hz), 3.66-3.63 (m, 1 H, H-6"), 3.59 (dd, 1 H, H-5', *J* = 6.2, 10.7 Hz), 3.52 (dd, 1 H, H-6", *J* = 5.8, 9.8 Hz), 2.20-1.99 (m, 3 H, H-5"×2, H-4"), 1.51 (m, 1 H, thexyl-CH), 1.56, 1.42, 1.34, 1.21 (each s, each 3 H, *i*-Pr-Me), 0.79-0.74 (s, 21 H, *t*-Bu, thexyl-Me), -0.02--0.16 (m, 12 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 154.16, 153.12, 152.17, 151.95, 135.72, 132.62, 128.89, 128.73, 128.68, 128.50, 114.59, 112.87, 91.81, 88.20, 84.04, 83.13, 82.10, 80.81, 68.77, 64.26, 63.56, 63.34, 47.01, 34.45, 32.94, 28.16, 27.53, 26.27, 26.16, 26.08, 25.77, 25.39, 20.63, 18.82, -3.32. *Anal.* calcd for $C_{44}H_{68}BrN_5O_9Si_2$: C 55.80, H 7.24, N 7.39. Found: C, 55.80; H, 7.28; N, 7.10.

8-Bromo-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(dimethylthexylsilyloxymethyl)cyclopentyl]-5'-*O*-4,4'-dimethoxytrityl-2',3'-*O*-isopropylidene-*N*^{6}-trichloroacetyladenosine (7c). To a solution of **14** (1.04 g, 1.52 mmol) and Et_3N (423 μL, 3.04 mmol) in CH_2Cl_2 (5 mL) was added CCl_3COCl (254 μL, 2.28 mmol) at 0 °C, and the mixture was stirred at the same temperature for 3 min. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. A mixture of the residue, 18-crown-6 (402 mg, 1.52 mmol), and K_2CO_3 (210 mg, 1.52 mmol) in DME (4 mL) was heated under reflux for 1 h. To the mixture was added a solution of **8** (645 mg, 1.52 mmol) in DME (1 mL) was added at 50 °C, and the resulting mixture was stirred at the same temperature for 21 h. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 5:1 then EtOAc) to give **7c** (263 mg, 17%) as solids, and **14** (636 mg, 61%) was recovered: UV (MeOH) λ_{max} 317, 283 nm; FAB-MS (positive) *m/z* 1144 (MH^+, 1%), 1146 (MH^+, 3%), 1148 (MH^+, 2%); FAB-HRMS calcd for $C_{53}H_{66}BrCl_3N_5O_{10}Si$ 1144.2828, found 1144.2800; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1 H, H-2), 7.38-6.77 (m, 13 H, DMTr-), 6.14 (d, 1 H, H-1', *J* = 2.3 Hz), 5.38 (dd, 1 H, H-2', *J* = 2.3, 6.4 Hz), 5.19 (dd, 1 H, H-2", *J* = 5.1, 6.9 Hz), 5.03 (dd, 1 H, H-3', *J* = 3.7, 6.4 Hz), 4.61-4.55 (m, 1 H, H-1"), 4.52 (dd, 1 H, H-3", *J* = 6.3, 6.4 Hz), 4.41 (m, 1 H, H-4'), 3.81 (m, 7 H, -OMe×2, H-6"), 3.61 (dd, 1 H, H-6", *J* = 7.3, 9.9 Hz), 3.32 (dd, 1 H, H-5', *J* = 6.4, 9.8 Hz), 3.26 (dd, 1 H, H-5', *J* = 5.6, 9.8 Hz), 2.58 (m, 1 H, H-5"), 2.34-2.25 (m, 2 H, H-5", H-4"), 1.61 (m, 1 H, thexyl-CH), 1.60, 1.53, 1.37, 1.27 (each s, each 3 H, *i*-Pr-Me), 0.88-0.82 (m, 12 H, thexyl-Me), 0.09 (s, 6 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 168.18 (C=O), 149.54 (C6), 147.01 (C4), 145.78 (C2), 144.54, 136.24, 135.72, 130.28, 130.06, 128.37, 128.19, 127.54 (C8), 123.50 (C5), 114.63 (-$C(CH_3)_2$), 113.48 (-$C(CH_3)_2$), 113.33, 91.38 (C1'), 86.09 (C4'), 83.31 (C2'), 81.89 (C2"), 81.46 (C3'), 81.01 (C3"), 69.33 (C1"), 64.19 (C6"), 63.43 (C5'), 55.27 (-OMe), 46.67 (C4"), 34.19, 33.03 (C5"), 27.6, 27.24, 25.45, 25.18, 20.36, 18.52, -3.47; NOE (400 MHz, $CDCl_3$), irradiated H-2, observed H-1" (12.0%), H-2" (4.0%).

8-Bromo-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(dimethylthexylsilyloxymethyl)-cyclopentyl]-2',3'-*O*-isopropylidene-*N*^{6}-trichloroacetyladenosine (17). A solution of **7c** (483 mg, 0.42 mmol) in THF (1 mL) and AcOH (4 mL) was stirred at room temperature for 6 h and then at 60 °C for 20 min. EtOAc and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 3:2) to give **17** (227 mg, 64%) as solids; UV (MeOH) λ_{max} 317 nm; FAB-MS (positive) *m/z* 842 (MH^+, 12%), 844 (MH^+, 19%), 846 (MH^+, 11%); FAB-HRMS calcd for $C_{32}H_{48}BrCl_3N_5O_8Si$ 842.1521, found 842.1500; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.07 (s, 1 H, H-2), 6.07 (d, 1 H, H-1', *J* = 5.0 Hz), 5.21 (dd, 1 H, H-2", *J* = 5.6, 6.5 Hz), 5.13 (dd, 1 H, H-2', *J* = 5.0, 5.9 Hz), 5.03 (dd, 1 H, H-3', *J* = 1.4, 5.9 Hz), 4.84-4.78 (m, 1 H, H-1"), 4.55 (dd, 1 H, H-3", *J* = 5.4, 6.5 Hz), 4.45 (s, 2 H, H-4', -OH), 3.91 (d, 1 H, H-5', *J* = 12.3 Hz), 3.81-3.75 (m, 2 H, H-5', H-6"), 3.63 (dd, 1 H, H-6", *J* = 6.6, 9.9 Hz), 2.50 (m, 1 H, H-5"), 2.39-2.32 (m, 2 H, H-5", H-4"), 1.58 (m, 1 H, thexyl-CH), 1.65, 1.53, 1.37, 1.28 (each s, each 3 H, *i*-Pr-Me), 0.90-0.84 (m, 12 H, thexyl-Me), 0.09 (s, 6 H, SiMe); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 168.57 (C=O), 148.77 (C6), 146.20 (C4), 146.13 (C2), 127.15 (C8),

123.94 (C5), 114.61 (-*C*(CH_3)$_2$), 113.64 (-*C*(CH_3)$_2$), 95.31 (C1'), 93.19 (C4'), 85.59 (C2'), 82.95 (C2"), 81.63 (CCl_3), 81.02 (C3'), 80.94 (C3"), 68.73 (C1"), 63.92 (C6"), 62.98 (C5'), 46.26 (C4"), 34.19, 33.10 (C5"), 27.68, 27.56, 25.41, 25.20, 20.36, 18.53, -3.45, -3.50.

8-Bromo-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(hydroxymethyl)cyclopentyl]-2',3'-*O*-isopropylidene-N^6-trichloroacetyladenosine (18). A solution of **17** (263 mg, 0.31 mmol), AcOH (178 µL, 3.12 mmol), and TBAF (1 M in THF, 624 µL, 0.624 mmol) in THF (3 mL) was stirred at room temperature for 4 days. $CHCl_3$ and water were added and the mixture was partitioned. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/EtOH, 15:1) to give **18** (204 mg, 93%) as solids: UV (MeOH) λ_{max} 317 nm; FAB-MS (positive) *m/z* 700 (MH^+, 12%), 702 (MH^+, 20%), 704 (MH^+, 15%); FAB-HRMS calcd for $C_{24}H_{30}BrCl_3N_5O_8$ 700.0334, found 700.0369; ^{1}H-NMR (500 MHz, $CDCl_3$ + D_2O) δ 8.16 (s, 1 H, H-2), 6.07 (d, 1 H, H-1', *J* = 5.0 Hz), 5.29 (m, 1 H, H-2"), 5.13 (dd, 1 H, H-2', *J* = 5.0, 5.8 Hz), 5.02 (dd, 1 H, H-3', *J* = 1.7, 5.8 Hz), 4.81-4.78 (m, 1 H, H-1"), 4.68 (m, 1 H, H-3"), 4.45 (d, 1 H, H-4', *J* = 1.8), 3.91 (dd, 1 H, H-5', *J* = 1.8, 12.5 Hz), 3.77 (m, 3 H, H-5', H-6"×2), 2.62 (m, 1 H, H-5"), 2.40-2.32 (m, 2 H, H-5", H-4"), 1.65, 1.54, 1.37, 1.29 (each s, each 3 H, *i*-Pr-Me); ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 169.49 (C=O), 149.74 (C6), 147.49 (C2), 147.19 (C4), 128.16 (C8), 124.87 (C5), 115.58 (-*C*(CH_3)$_2$), 114.70 (-*C*(CH_3)$_2$), 94.07 (C1'), 86.52 (C4'), 83.88 (C2'), 82.86 (C3"), 82.73 (C2"), 81.92 (C3'), 70.10 (C1"), 64.91 (C6"), 63.87 (C5'), 47.32 (C4"), 33.14 (C5"), 28.54, 28.47, 26.33, 26.11; NOE (400 MHz, $CDCl_3$) irradiated H-2, observed H1" (17.3%), H-5" (1.5%).

8-Chloro-1-[(1*R*,2*S*,3*R*,4*R*)-2,3-isopropylidenedioxy-4-(phosphonoxymethyl)cyclopentyl]-2',3'-*O*-isopropylidene-N^6-trichloroacetyl-5'-*O*-phosphonoadenosine (19). $POCl_3$ (129 µL, 1.4 mmol) was added to a solution of **18** (20 mg, 0.028 mmol) in $PO(OEt)_3$ (2 mL) at 0 °C, and the mixture was stirred at the same temperature for 6 h. The reaction was quenched by aqueous saturated $NaHCO_3$ (5 mL), and pH of the resulting mixture was adjusted to about 5 with AcOH. The resulting mixture was diluted with water (50 mL) and applied to a DEAE-Sephadex A-25 column (HCO_3^- form, 1.8 x 8 cm). After washing with water (100 mL), the column was developed using a linear gradient of 0.1 N triethylammonium acetate (TEAA) buffer (pH 8.3) to 0.5 M TEAA buffer (pH 8.3). Fractions were analyzed by HPLC [YMC-ODS-M80, 4.6×150 mm; 5-80% MeCN in 0.1 N TEAA Buffer (pH 8.3), 1.0 mL/min; 254 and 317 nm] and the appropriate fractions were evaporated under reduced pressure, and then excess TEAA was coevaporated with water. The residue was freeze-dried to give triethylammonium salt of **19** (12 mg, 43%) as solids: UV (MeOH); λ_{max} 317, 207 nm; (acid) 317 nm; (base) 317, 209 nm; FAB-MS (negative) *m/z* 814 [(M-H)$^-$, 36%], 816 [(M-H)$^-$, 50%], 818 [(M-H)$^-$, 26%]; FAB-HRMS calcd for $C_{24}H_{30}Cl_4N_5O_{14}P_2$ 814.0018, found 814.0003; ^{1}H-NMR (500 MHz, D_2O) δ 8.88 (s, 1 H, H-2), 6.48 (d, 1 H, H-1', *J* = 2.0 Hz), 5.82 (dd, 1 H, H-2', *J* = 2.0, 6.3 Hz), 5.43-5.36 (m, 3 H, H-3',H-1", H-2"), 4.92-4.76 (m, 1 H, H-3"), 4.16-3.96 (m, 4 H, H-5'×2, H-6"×2), 3.24 (q, 12 H, CH_3CH_2N-, *J* = 7.1 Hz), 2.58 (m, 3 H, H-4', H-5"×2), 1.69, 1.63, 1.49, 1.38 (each s, each 3 H, *i*-Pr-Me), 1.32 (t, 18 H, CH_3CH_2N-, *J* = 7.1 Hz); ^{13}C-NMR (67.8 MHz, D_2O) δ 171.05 (C=O), 156.26 (C6), 151.77 (C4), 150.83 (C2), 144.87 (C8), 125.21 (C5), 118.02 (-*C*(CH_3)$_2$), 117.41 (-*C*(CH_3)$_2$), 92.72 (C1'), 85.95 (C4'), 85.52 (C2'), 84.20 (C2"), 83.81 (C3", C3'), 70.12 (C1"),

68.77 (C6"), 67.12 (C5'), 61.51, 47.52 (C4"), 46.82, 35.65 (C5"), 28.80, 27.15, 11.07; NOE (400 MHz, $CDCl_3$) 9.1% (H-1"-H-2); ^{31}P-NMR (125 MHz, D_2O) δ ; 0.72, 0.68.

Cyclic 8-Chloro-N^6-trichloroacetyl-ADP-carbocyclic-ribose Diacetonide (20). To a solution of EDC·HCl (9.4 mg, 0.048 mmol) in NMP (6 mL) was added slowly a solution of **19** (24.7 mg, 0.024 mmol) in NMP (1 mL) at 80 °C, and the resulting mixture was stirred at the same temperature for 2 min. Compound **22** (sodium salts, 56 mg, 0.074 mmol) was dissolved in NMP (11 mL) by heating. EDC (21 mg, 0.11 mmol) was added to the solution of **22**, and the mixture was stirred at 50 °C for 60 h. After cooling the mixture with ice-bath, the mixture was diluted with water (50 mL). The solution was applied to a DEAE-Sephadex A-25 column (HCO_3^- form, 1.8 x 6 cm). The column was washed with water (300 mL), and developed using a linear gradient of 0 to 0.4 M TEAB buffer (pH 8.3, 200 mL). Fractions were analyzed by HPLC [YMC-ODS-M80, 4.6×150 mm; 5-80% MeCN in 0.1 N TEAA Buffer (pH 8.3), 1.0 mL/min; 254 and 317 nm] and the appropriate fractions (were evaporated under reduced pressure, and then excess TEAB was coevaporated with water. Counter cations were exchanged for sodium with a Diaion WK-20 resin column (Na^+ form, 1.2 x 5 cm, developed by water). The eluate was evaporated under reduced pressure, and the residue was freeze-dried to give **20** (sodium salt, 2.4 mg, 10%) as solids: UV (MeOH); λ_{max} 315 nm; FAB-MS (negative) *m/z* 796 [(M-H)$^-$, 19%], 798 [(M-H]), 26%), 800[(M-H)$^-$, 12%]; FAB-HRMS calcd for $C_{24}H_{28}Cl_4N_5O_{13}P_2$ 795.9913, found 795.9929; FAB-MS (positive) *m/z* 798 (MH^+, 27%), 800 (MH^+, 34%), 802 (MH^+, 19%); FAB-HRMS calcd for $C_{24}H_{30}Cl_4N_5O_{13}P_2$ 798.0069, found 798.0080; ^{1}H-NMR (500 MHz, D_2O) δ 9.09 (s, 1 H, H-2), 6.46 (s, 1 H, H-1'), 5.78 (d, 1 H, H-2', J = 6.2 Hz), 5.57 (m, 1 H, H-3'), 5.40 (m, 1 H, H-1"),4.84-4.79 (m, 2 H, H-2", H-3"), 4.63 (m, 1 H, H-4'), 4.22 (m, 1 H, H-5' or H-6"), 4.07 (m, 2 H, H-5 or H-6"), 3.87 (m, 1 H, H-5' or H-6"), 3.07 (m, 1 H, H-5" or H-4"), 2.87 (m, 1 H, H-5" or H-4"), 2.66 (m, 1 H, H-5" or H-4"), 1.68, 1.60, 1.48, 1.37 (each s, each 3 H); ^{13}C-NMR (67.8 MHz, D_2O) δ 170.09 (C=O), 156.30 (C6), 153.01 (C4, C2), 144.91 (C8), 124.96 (C5), 117.37 (-$C(CH_3)_2$), 113.99 (-$C(CH_3)_2$), 93.89 (C1'), 90.82 (C4'), 86.66 (C2'), 84.52 (C2"), 86.08 (C3'), 84.52 (C2", C3"), 70.69 (C1"), 68.74 (C6"), 66.82 (C5'), 46.87 (C4"), 31.84 (C5"), 28.73, 28.65, 26.99, 26.45; NOE (400 MHz, D_2O) irradiated H-2, observed H-5" (7.2%), H-2" (2.4%), H-5' (2.0%), H-1' (1.86%); ^{31}P-NMR (125 MHz, D_2O) δ ; -10.51, -10.57 (each d, J = 12.5 Hz).

Acknowledgment.

This investigation was supported in part by a Grant from the Ministry of Education, Science, Sports, and Culture of Japan. We are grateful to Prof. Barry. V. L. Potter (University of Bath) for his helpful discussion.

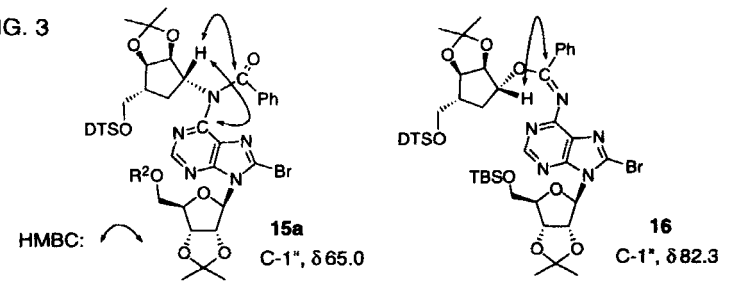
REFERENCES AND NOTES

1. Part 191: Nomura, M.; Shuto, S.; Matsuda, A. submitted.
2. (a) Clapper, D. L.; Walseth, T. F.; Dargie, P. J.; Lee, H. C. *J. Biol. Chem.* **1987**, *262*, 9561-9568. (b) Galione, A. *Science* **1993**, *259*, 325-326. (c) Lee, H. C.; Galione A.; Walseth, T. F. *Vitamines Hormones* **1994**, *48*, 199-257. (d) Lee, H. C.; Aarhus, R. *Biochim. Biophya. Acta* **1993**, *1164*, 68-74.
3. (a) Walseth, T. F.; Lee, H. C. *Biochem. Biophys. Acta* **1993**, *1178*, 235-242. (b) Lee, H. C.; Aarhus, R.; Walseth, T. F. *Science* **1993**, *261*, 352-355. (c) Graeff, R. M.; Walseth, T. J.;

Fryxell, K.; Branton, W. D.; Lee, H. C. *J. Biol. Chem.* **1994**, *269*, 30260-30267. (d) Zhang, F-J.; Sih, C. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1701-1706. (e) Zhang, F-J.; Gu, Q-M.; Jing, P.C.; Sih, C. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2267-2272. (f) Zhang, F-J.; Yamada, S.; Gu, Q-M.; Sih, C. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1203-1208. (g) Zhang, F-J.; Sih, C. J. *Tetrahedron. Lett.* **1995**, *63*, 9289-9292. (h) Zhang, F-J.; Sih, C. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2311-2316. (i) Ashamu, G. A.; Galione, A.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1995**, 1359-1356. (j) Bailey, B. C.; Fortt, S. M.; Summerhill, R. J.; Galione, A.; Potter, B. V. L. *FEBS Lett.* **1996**, *379*, 227-230. (k) Bailey, V. C.; Sethi, J. K.; Fortt, S. M.; Galione, A.; Potter, B. V. L. *Chem. Biol.* **1997**, *4*, 51-60. (l) Bailey, V. C.; Sethi, J. K.; Galione, A.; Potter, B. V. L. *J. Chem. Soc. Chem. Commun.* **1997**, 695-696. (m) Sethi, J. K.; Empson, R. M.; Bailey, V. C.; Potter, B. V. L. Galione, A. *J. Biol. Chem.* **1997**, *272*, 16358-16363. (n) Ashamu, G. A.; Sethi, J. K.; Galione, A.; Potter, B. V. L. *Biochemistry* **1997**, *36*, 9509-9517. (o) Zhang, F-J.; Gu, Q-M.; Sih, C. J. *Bioorg. Med. Chem.*. **1999**,7, 653-664.

4. (a) Shuto, S; Shirato, M; Sumita, Y; Ueno, Y; Matsuda, A. *J. Org. Chem.* **1998**, *52*, 1986-1994. (b) Fukuoka, M.; Shuto, S.; Minakawa, N.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1999**, *40*, 5361-5364.
5. Synthetic approaches to carbocyclic analogues of cADP-ribose by other groups have been published. Although an *N*-1-carbocyclic-inosine or –adenosine structure has been constructed, formation of the intramolecular pyrophosphate linkage has not been achieved. (a) Fortt, S.; Potter, B. V. L. *Tetrahedron Lett.* **1997**, *38*, 5371-5374. (b) Hutchinson, E. J.; Taylor, B. F.; Blackburn, G. M. *J. Chem. Soc. Chem. Commun.* **1997**, 1859-1860.
6. Shuto, S.; Shirato, M.; Sumita, Y.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1998**, *39*, 7341-7344.
7. Saenger, W. *Principals of nucleic acid structure*; Springer-Verlag, 1983.
8. (a) Travale, S. S.; Sobell, M. *J. Mol. Biol.* **1970**, 48, 109-123. (b) Ikehara, M.; Uesugi, S.; Yoshida, K. *Biochemistry* **1972**, 11, 830-836.
9. Ikehara, M.; Uesugi, S.; Kaneko, M. *Tetrahedron* **1970**, *26*, 4251-4259.
10. When H-2 of the purine ring in **15a** or **16** was irradiated, an NOE was not observed, which showed that these compounds were not *N*-1-carbocyclic products. The carbocyclic unit-attached positions in **15a** and **16** were determined by correlations in their HMBC spectra and the chemical shifts of C-1" in their ^{13}C NMR spectra in FIG. 3.

FIG. 3



11. Yoshikawa, M.; Kato, T.; Takenishi. T. *Bull, Chem. Soc. Jpn.* **1969**, *42*, 3505-3508.
12. In this reaction, the substrate **19** was mostly decomposed, and **20** was the only product isolated.
13. Wada, T.; Inageda, T.; Sekine, M. *Nucleosides Nucleotides* **1995**, *14*, 1301-1341.
14. Gu, Q-M.; Sih, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 7481-7486.